Relationship of Apolipoproteins and Subclinical Cardiovascular Risk in Children and Adolescents

A THESIS
SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA
BY

Madeline Anne Czeck

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Donald R. Dengel, Ph.D.

May 2020

ACKNOWLEDGMENTS

I am thankful to those who have contributed their time, efforts, and support towards the completion of this thesis. First, I am grateful to have been given the opportunity to prepare this thesis under the advisement of Dr. Donald Dengel and my committee members, Dr. Justin Ryder and Dr. Aaron Kelly. Thank you to these three individuals for providing excellent mentorship and feedback throughout the entirety of this project. Next, I would like to thank Dr. Kyle Rudser and Elise Northrop from the University of Minnesota Biostatistical Design and Analysis Center for their contributions to the study designs, analyses, and outcomes of this thesis. Additionally, I wish to thank Erica Roelofs and Nicholas Evanoff for their help and support during the past two years. Lastly, a special thanks to my family for their constant encouragement and support throughout this process.

ABSTRACT

INTRODUCTION: Apolipoproteins play a role in regulating cholesterol transportation and clearance with each apolipoprotein having a protective or atherogenic role. However, previous literature in youth have insufficiently described the relationship between each apolipoprotein variable and subclinical cardiovascular risk. Hence, the purpose of this study was to examine the association of apolipoproteins with measures of vascular structure and function in children and adolescents.

METHODS: A cross-sectional study of 338 youth (160 males, 178 females; mean age = 13.0±2.8 years) with a range of adiposities from normal body weight to severe obesity. Apolipoproteins (AI, AII, B_{100}, CII, CIII, and E) were measured via human apolipoprotein magnetic bead panel. Ultrasound imaging of the carotid artery was used to measure carotid intima-media thickness (cIMT), carotid cross-sectional compliance (cCSC), carotid diameter compliance (cDC), carotid cross-sectional distensibility (cCSD), carotid diameter distensibility (cDD), and carotid incremental elastic modulus (cIEM). Applanation tonometry assessed pulse wave velocity (PWV). Dual X-ray absorptiometry measured total body fat percent. Linear regression models were adjusted for Tanner stage, sex, and race with further adjustments for body fat percent. Data are presented as mean [95% CI] with Holm's-adjusted p-values to account for multiple testing. All apolipoproteins were scaled to 10 μg/mL and apolipoprotein ratios were scaled to 0.1 μg/mL.

RESULTS: Prior to accounting for multiple testing, apolipoprotein CIII:CII ratio was positively associated with cIMT (0.001 [0, 0.003], p = 0.033), but failed to maintain significance after p-value correction. Otherwise, there were no significant associations

between any apolipoprotein and cIMT, cCSC, cDC, cCSD, cDD, and cIEM in the presence or absence of body fat percent. There were significant positive associations between PWV and apolipoproteins: AII (0.04 m/sec [0.02, 0.06], p = 0.046) and E (0.16 m/sec [0.08, 0.24], p = 0.012). After adding body fat percent to the models, PWV remained positively associated with higher levels of apolipoproteins E (0.16 m/sec [0.08, 0.24], p = 0.01).

CONCLUSIONS: These findings suggest higher levels of apolipoprotein E are associated with higher arterial stiffness (PWV) in pediatrics, both in the presence and absence of excess body fat.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

APO: Apolipoprotein

BMI: Body Mass Index

cCSC: carotid Cross-Sectional Compliance

cCSD: carotid Cross-Sectional Distensibility

cDC: carotid Diameter Compliance

cDD: carotid Diameter Distensibility

cIEM: carotid Incremental Elastic Modulus

cIMT: carotid Intima-Media Thickness

ECG: Electrocardiogram

PWV: Pulse Wave Velocity

CHAPTER 1: INTRODUCTION

The pathophysiology leading to cardiovascular disease begins in childhood and is accelerated by advancing age, male sex, dyslipidemia, hypertension, obesity, and tobacco smoking (Berenson et al., 1998; Dengel & Bronas, 2010; O'Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002; Rainwater et al., 1999). Specifically, maintaining normal levels of cholesterol is one of the many factors essential for the prevention of cardiovascular disease. Apolipoproteins (APO) are formed predominately in the liver and intestine, and they play an essential role in the production and transportation of cholesterol around the body (Donma & Donma, 1989). There are a variety of APOs (e.g., AI, AII, B_{100}, CII, CIII, and E), with each type binding to either high-density lipoprotein, low-density lipoprotein, very-low-density lipoprotein, or chylomicrons in the blood. Depending on the concentrations of each APO, there may be more atherogenic properties (i.e., B_{100}, CIII, E) or protective properties (i.e., AI, AII, CII), thereby affecting vascular structure and function (Dengel & Bronas, 2010). Notably, decreases in endothelial function play an essential role in the progression of atherosclerosis and is considered a marker of subclinical cardiovascular disease (Anderson et al., 1995; Vrints, Bult, Bosmans, Herman, & Snoeck, 1992; Yasue et al., 1990; Zeiher, Drexler, Wollschläger, & Just, 1991). Therefore, APOs may have a direct impact on the risk of cardiovascular disease.

Previous literature on the association of APO variables and cardiovascular disease risk factors have primarily been in adults (Gerber, Goldbourt, Cohen, & Harats, 2002; Huang et al., 2013; Ooi, Barrett, Chan, & Watts, 2008; Sacks et al., 2000; Schaefer et al., 1994; Steffen et al., 2017) while recent literature in children has primarily been focused

on differences in APO-AI and APO-B_{100} concentrations. Some studies report associations of APO-AI and APO-B_{100} across body mass index (BMI) in children (Hobkirk et al., 2014; Krekoukia et al., 2007; Teixeira, Sardinha, Going, & Lohman, 2001). Specifically, these studies report lower levels of APO-AI and higher levels of APO-B_{100} in children with obesity compared to their counterparts without obesity (Krekoukia et al., 2007; Teixeira et al., 2001). This is important since vascular structure and function are affected by childhood obesity and may increase the risk of cardiovascular disease (Burke et al., 2008; Kotis et al., 2006; Steinberg et al., 1996; Tounian et al., 2001; Williams et al., 2005; Woo et al., 2004). Previous studies in children have explored the association of APO-AI and APO-B_{100} and carotid intima-media thickness (cIMT). They have reported that adulthood cIMT was inversely related to APO-AI and directly related with APO-B_{100} (Frontini et al., 2008; Kallio et al., 2010; Juonala et al., 2008). To date, few studies have examined APO concentrations in relation to vascular function in children (Juonala et al., 2008; Tounian et al., 2001). Particularly, these researchers examined the relationship between APO-AI and flow-mediated dilation in children, a measure of endothelial dysfunction. They observed a strong positive correlation between flow-mediated dilation and APO-AI concentration in both children with normal weight and children with obesity (Juonala et al., 2008; Tounian et al., 2001). These results indicated a possible relationship between low APO-AI levels and endothelial dysfunction in children. Therefore, while previous studies have explored APO-AI and APO-B_{100} concentrations in children and adolescents, these studies have not examined other APO concentrations (i.e., AII, CII, CIII, E). Consequently, more research on the APO variables (i.e., AI, AII, B_{100}, CII, CIII, E) in children and adolescents and their potential association with subclinical

cardiovascular risk is warranted. The purpose of this study was to examine the association of APOs with measures of vascular structure and function among children and adolescents.

The following chapters of this thesis include a literature review, methodological explanations, results summary, discussion, and conclusion.

Chapter two summarizes the current literature related to apolipoprotein function. Measurement techniques to examine subclinical cardiovascular disease will be described. Additionally, the relationship between apolipoproteins and cardiovascular disease in adults and children will be explored.

Chapter three includes details on the study's methodology including information on the study population; procedures and measurement techniques, including anthropometric, blood pressure, blood assay, and vascular assessments; and statistical analyses.

Chapter four summarizes the results of the study. Associations of each apolipoprotein variable between measures of vascular structure and function.

Chapter five includes a discussion of study results. Findings are presented in regard to previously published studies related to the current topic.

Chapter six provides final conclusions as well as research implications of the study's findings.

CHAPTER 2: LITERATURE REVIEW

Apolipoproteins

What Are Apolipoproteins?

APOs are major proteins that assist in the production, transportation, and clearance of cholesterol. There are a variety of APOs (e.g., AI, AII, B_{100}, CII, CIII, and E), with each type binding to either high-density lipoprotein, low-density lipoprotein, very-low-density lipoprotein, or chylomicrons (Donma & Donma, 1989; Schaefer, Eisenberg, & Levy, 1978). In lipoprotein metabolism, APOs have a variety of roles. These include acting as structural proteins for lipoproteins, cofactors for enzymes, and ligands for binding to cell-surface receptors (Rader et al., 1994).

APO-AI is the major protein component of high-density lipoprotein and minor component of chylomicrons and very-low-density lipoproteins. It assists in the activation of lecithin-cholesterol acyltransferase (Schaefer et al., 1978; Tian & Fu, 2011). APO-AI plays a major role in reverse transport of cholesterol to the liver. This process is done by APO-AI removing excess cholesterol from tissues and incorporating it into high-density lipoprotein cholesterol (Erdeve, Simsek, Dallar, & Biyikli, 2010). Similarly, APO-AII is the second most abundant protein in high-density lipoprotein. However, the specific roles of APO-AII in high-density lipoprotein metabolism are complex and have been controversial (Tailleux, Duriez, Fruchart, & Clavey, 2002). The roles for APO-AII include acting as an activator of hepatic lipase, inhibitor of cholesteryl ester transfer protein, and inhibitor of lecithin-cholesterol acyltransferase (Tian & Fu, 2011, Rader et al., 1994). Inhibition of lecithin-cholesterol acyltransferase may be a potential harmful effect. Whereas inhibition of cholesteryl ester transfer protein and activation of hepatic

lipase may be a potential beneficial effect. Therefore, the opposing roles in lipoprotein metabolism provide conflicting evidence in regard to whether APO-AII is antiatherogenic or proatherogenic.

On the other hand, APO-B_{100} is a major protein component of low-density lipoproteins, very-low density lipoproteins, and chylomicrons. There is only one APO-B_{100} particle per particle—very-low-density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein—with these particles having atherogenic properties (Walldius & Jungner, 2007). The major role of APO-B_{100} is triglyceride transport (Schaefer et al., 1978). In metabolism, APO-B_{100} combines with cholesterol and triglycerides to produce very-low-density lipoproteins. Likewise, the APO-B_{100}:AI ratio reflects the cholesterol balance. Particularly, the ratio between potentially atherogenic lipoproteins and antiatherogenic lipoproteins (Walldius & Jungner, 2007). However, higher levels of APO-B100 does not mean low levels of APO-AI

In addition, APO-CII and APO-CIII are protein components of chylomicrons, very-low-density lipoprotein, and high-density lipoprotein. APO-CII plays a role in lipoprotein lipase activation (Kei, Filippatos, Tsimihodimos, & Elisaf, 2012; Schaefer et al., 1978; Tian & Fu, 2011). On the other hand, APO-CIII plays a role in inhibition of lipoprotein lipase, lipoproteins binding to receptors, and regulates the uptake of triglyceride-rich lipoprotein remnants via hepatic receptors (Ooi et al., 2008; Rader et al., 1994; Schaefer et al., 1978; Tian & Fu, 2011).

Lastly, APO-E is a protein component of very-low-density lipoprotein and high-density lipoprotein. It is a ligand for binding to low-density lipoprotein receptor (Rader et al., 1994). In addition, APO-E assists as a ligand for clearance of the lipoproteins

containing APO-B. However, it does not aid in the clearance of low-density lipoproteins. Additionally, the APO-E gene is polymorphic with three alleles (i.e., ε2, ε3, ε4) (Davignon, Gregg, & Sing, 1988; Utermann, Hardewig, & Zimmer, 1984). Previous research has reported that allele phenotype impacts cholesterol levels. For example, ε2 has been observed to correspond with lower low-density lipoproteins levels, where ε4 has been observed to correspond with higher low-density lipoprotein levels. Also, the ε4 allele has been observed to increase the risk of cardiovascular disease compared to other individuals expressing the ε2 and ε3 allele (Davignon et al., 1988; Kolovou, Daskalova, & Mikhailidis, 2003; Song, Stampfer, & Liu, 2004; Utermann et al., 1984). Ultimately, the ε4 allele could favor the development of atherosclerosis (Doliner et al., 2018; Slooter et al., 2001; Volcik et al., 2006).

Effect of Age on Apolipoproteins

Previous literature has reported inconsistent results on the effect of age on each APO. Concentrations of APO-AI have been reported to increase (Anagnostis, Stevenson, Crook, Johnston, & Godsland, 2016, Sniderman et al., 2016), decrease (Anagnostis et al., 2016), and remain the same with increasing age (Sakurabayashi, Saito, Kita, Matsuzawa, & Goto, 2001). However, it has been noted that women consistently have higher APO-AI concentrations compared to men (Anagnostis et al., 2016; Sakurabayashi et al., 2001). Whereas, APO-AII concentrations have been reported to increase with age in women and decrease with age in men (Anagnostis et al., 2016; Sakurabayashi et al., 2001). Similarly, APO-B_{100} concentrations have been reported to either increase with increasing age

(Anagnostis et al., 2016; Sakurabayashi et al., 2001) or remain the same (Sniderman et al., 2016). Due to the conflicting results of the impact of age with APO-AI and APO-B_{100}, the APO-B_{100}:AI has also been reported to either decrease or increase with age (McQueen et al., 2008; Sakurabayashi et al., 2001; Sniderman et al., 2007). Previous research has observed the levels of both APO-CII and APO-CIII to increase with age (Sakurabayashi et al., 2001). Lastly, a previous study reported an increase in APO-E with age. Also, women had higher concentrations of APO-E compared to men regardless of age (Sakurabayashi et al., 2001). While these previous studies explore the relationship of age on APO concentrations, these studies were conducted in individuals over 20 years old, with participants predominatley being middle age and older. Therefore, more research is needed to determine the effect of age, including children and adolescents.

Effect of Body Fat on Apolipoproteins

Previous research conducted exploring the relationship between body fat and APO concentrations have predominately been in APO-AI, APO-B_{100}, APO-CII, and the APO-B_{100}:AI ratio (Anagnostis et al., 2016; Kallio et al., 2010; Karabouta, Papandreou, Makedou, Rousso, & Athanassiadou, 2016). Notably, children with obesity had lower APO-AI levels compared to their normal weight peers (Karabouta et al., 2016). Previous research has also reported that a higher BMI was associated with higher APO-B_{100} and APO-B_{100}:AI ratio (Anagnostis et al., 2008). Other research exploring the effect of body fat and APOs, specifically APO-AI and APO-$B_{100,}$ have been conducted in individuals with and without the metabolic syndrome (Alemzadeh & Kichler, 2018; Erdeve et al., 2010). Individuals with the metabolic syndrome had significantly lower levels of APO-AI

and higher levels of APO-B_{100} and APO-B_{100}:AI ratio compared to individuals without the metabolic syndrome (Alemzadeh & Kichler, 2018; Erdeve et al., 2010). Therefore, body fat may have an effect on the concentrations of each APO. However, more research is needed to explore how body fat impacts all of the APO variables.

Cardiovascular Disease

Arterial stiffness is an important factor in the progression of cardiovascular disease. In addition, two major determinants of arterial stiffness are collagen and elastin. Specifically, vascular stiffness occurs from an overproduction of abnormal collagen and reduced normal elastin (Laurent et al., 2006). While there are molecular and cellular factors of arterial stiffness, there are also clinical conditions associated with increased arterial stiffness. These include advancing age, physiological conditions (i.e., lack of physical activity), genetic background (i.e., parental history of myocardial infarction), cardiovascular risk factors (i.e., obesity, smoking, hypertension), and cardiovascular diseases (i.e., coronary heart disease) (Laurent et al., 2006). Therefore, understanding factors that affect arterial stiffness is important because arterial stiffness is a subclinical marker of cardiovascular risk.

Effect of Age on Cardiovascular Disease

To determine the effect of age, especially early studies in children, autopsy studies have been the primary sources for showing the progression of cardiovascular disease. Autopsy studies have shown that fatty streaks in the coronary arteries increase with age (Berenson et al., 1998). Importantly, these studies have shown that

atherosclerosis begins in childhood. In addition, while atherosclerosis begins in childhood, it progresses with age. Therefore, there is an increased risk of atherosclerosis and cardiovascular disease with increasing age (Sniderman et al., 2016). This is due to an increase in collagen while there is a subsequent decrease in elastic fibers (Laurent et al., 2006). Thus, there is a worsening of endothelial dysfunction—a decrease in the ability of the artery to constrict or dilate in response to various physical and chemical stimuli (Dengel & Bronas, 2010). In subclinical atherosclerosis, endothelial dysfunction may occur prior to the thickening of the vascular wall (Kallio et al., 2010). A decrease in function before structural changes is the most common progression of the pathophysiology of atherosclerosis.

Effect of Body Fat on Cardiovascular Disease

While age is a non-modifiable factor that impacts cardiovascular disease, body fat is another factor that affects cardiovascular disease. Autopsy studies have reported a positive correlation with atherosclerotic lesions and BMI (Berenson et al., 1998). Research in children and adolescence exploring the relationship in body fat and non-invasive measures of cardiovascular disease, have reported that obesity in adolescence predicts a broad range of later health problems (Tounian et al., 2001). This includes early indications of decreased vascular structure and function. This was seen with a decrease in cross-sectional compliance and distensibility and flow-mediated dilation in the arteries (Tounian et al., 2001; Williams et al., 2005; Woo et al., 2004). In addition, there was an increase in carotid incremental elastic modulus (cIEM) and cIMT (Burke et al., 2008; Kotsis, Stabouli, Papamichael, & Zakopoulos, 2006; Tounian et al., 2001; Woo et al.,

2004). These risks for cardiovascular disease become more pronounced in individuals whom are overweight and with obesity (Karabouta et al., 2016). Therefore, increases in adiposity are associated with increased risk for cardiovascular disease (Lloyd-Jones et al., 2006).

Subclinical Measures of Atherosclerosis

The pathophysiology of atherosclerosis results in changes in both vascular structure and function that occur prior to the clinical diagnosis. However, there are various techniques to measure subclinical atherosclerosis non-invasively, which include cIMT, compliance (i.e., cross-sectional compliance [CSC], diameter compliance [DC]), distensibility (i.e., cross-sectional distensibility [CSD], diameter distensibility [DD]), cIEM, and pulse wave velocity (PWV). cIMT is a marker of structural atherosclerosis, with increased thickness considered an independent risk factor of cardiovascular disease (Juonala et al., 2008). In addition, arterial elasticity is determined by how well the artery can return to its previous shape after a deforming force or stress (Marlatt, Kelly, Steinberger, & Dengel, 2013). Arterial elasticity can be measured by compliance, distensibility, and IEM. Compliance is the change in volume prompted by a change in pressure and relates the artery as a hollow structure to provide information about the elasticity (Aggoun et al., 2000; Jani & Rajkumar, 2006; Reneman, Meinders, & Hoeks, 2005). Whereas distensibility is the change in arterial volume in contrast to the change in pressure (Reneman et al., 2005). Compared to compliance, distensibility relates more to wall stiffness relative to initial volume (Marlatt et al., 2013). While, IEM indicates

properties of the arterial wall independent of geometry (Aggoun et al., 2000; Tounian et al., 2001).

Pulse-wave velocity (PWV) measures arterial stiffness via applanation tonometry. The pulse wave is assessed between two sites (i.e., radial-carotid, femoral-carotid) in the line of pulse travel. The delay between the R-wave determined by the three-lead electrocardiogram (ECG) and the initial upstroke of the pulse wave is the point of reference for PWV quantification (Laurent et al., 2006; O'Rourke et al., 2002).

Apolipoproteins and Cardiovascular Disease

The risk of cardiovascular disease starts in childhood and progresses through adulthood. Similarly, a crucial role in the pathogenesis of atherosclerosis is the interaction between lipoproteins and endothelial cells (Bornaun et al., 2017). Depending on the concentrations of each APO, there may be more atherogenic properties (i.e., B_{100}, CIII, E) or protective properties (i.e., AI, AII, CII), thereby affecting vascular function. Specifically, decreases in vascular function play a critical role in the development of atherosclerosis (Anderson et al., 1995; Vrints et al, 1992; Yasue et al., 1990; Zeiher et al., 1991). However, APOs and APO ratios (i.e., B_{100}:AI) may be more sensitive and specific biomarkers compared to the use to lipids or lipoproteins for the assessment of cardiovascular disease risk (Donma & Donma, 1989).

Previous literature on the association of APO variables and cardiovascular disease risk factors have primarily been in adults (Gerber et al., 2002; Huang et al., 2013; Kei et al., 2012; Ooi et al., 2008; Sacks et al., 2000; Schaefer et al., 1994; Sniderman et al., 2007, 2016; Steffen et al., 2017; Stewart et al., 2007; Zivanovic et al., 2018). Previous

research in adults have reported that increased APO-B_{100} and APO-CII and decreased APO-AI and APO-E may play a role in the risk of cardiovascular disease (Kei et al., 2012; Sniderman et al., 2007; Stewart et al., 2007). In addition, previous literature in adults have explored the differences in APO concentrations in individuals who have had a myocardial infarction compared to healthy controls. These studies have reported that individuals who had a myocardial infarction had lower APO-AI and APO-AII and higher APO-B_{100} and APO-B_{100}:AI ratio compared to the controls (Kappelle, Gansevoort, Hillege, Wolffenbuttel, & Dullaart, 2011; McQueen et al., 2008; Pischon et al., 2005; Sharrett et al., 1994; Sniderman et al., 2016). Likewise, literature in adults have explored the association between APOs and cIMT. These researchers observed that patients with increased cIMT had lower mean APO-AI and higher mean APO-B_{100} and APO-B_{100}:AI ratio (Sharrett et al., 1994; Zivanovic et al., 2018). Notably, they reported the APO-B_{100}:AI ratio as the most significant predictor of increased cIMT (Wallenfeldt, Bokemark, Wikstrand, Hulthe, & Fagerberg, 2004; Zivanovic et al., 2018). Indicating that the ratio of APO-B_{100}:AI may be a strong predictor of atherosclerotic disease.

On the other hand, recent research in children has primarily been focused on differences in APO-AI and APO-B_{100} concentrations. Studies conducted have primarily explored the relationship between APO concentrations in children of parents who had either early onset coronary heart disease, history of cardiovascular disease, or myocardial infarction. These studies reported children of patients had lower concentrations of APO-AI and APO-AII, and higher APO-B_{100} and APO-B_{100}:AI ratio (Beigel et al., 1993; Bornaun et al., 2017; Freedman et al., 1986; Uiterwaal et al., 1996). In addition, previous studies in children have explored APO-AI and APO-B_{100} concentrations across BMI

(Hobkirk et al., 2014; Krekoukia et al., 2007; Teixeira et al., 2001). These studies report associations of APO-AI and APO-B_{100} with adiposity. Specifically, lower levels of APO-AI and higher levels of APO-B_{100} in children with obesity compared to their counterparts without obesity (Krekoukia et al., 2007; Teixeira et al., 2001). This is important since vascular structure and function are affected by childhood obesity and may increase the risk of cardiovascular disease (Dengel & Bronas, 2010).

To date, few studies have examined APO concentrations in relation to vascular function in children (Juonala et al., 2008; Tounian et al., 2001). Studies in children with obesity have reported a positive correlation between flow-mediated dilation and APO-AI concentration (Tounian et al., 2001). In addition, APO-AI was directly related to adulthood flow-mediated dilation (Juonala et al., 2008). Indicating a possible relationship between low APO-AI levels and endothelial dysfunction in children and the potential future effects of APO concentrations. While flow-mediated dilation is another measure of vascular function, specifically endothelial dysfunction, it was not a measure in the present study.

Likewise, few studies have explored the association between APOs and cIMT in children (Frontini et al., 2008; Juonala et al., 2008; Kallio et al., 2010). Specifically, the studies conducted by Juonala and colleagues (2008) and Frontini and colleagues (2008) were the most similar to the current study. Their purpose was to determine if APO-AI and APO-B_{100} measured in childhood predicts atherosclerosis risk in adulthood. Their participants were part of the Cardiovascular Risk in Young Finns Study and the Bogalusa Heart Study, respectively. They measured APO-AI and APO-B_{100} during childhood and cIMT in adulthood of the same participants. The first study observed APO-AI was

inversely related and APO-B_{100} and APO-B_{100}:AI ratio were directly related to adulthood cIMT (Juonala et al., 2008). Whereas the other study observed that APO-B_{100} and APO-B_{100}:AI ratio was a significant predictor of excess cIMT in adulthood (Frontini et al., 2008). Both studies concluded that these APOs measured in childhood predict increased cIMT in adulthood.

Therefore, while previous studies have explored APO-AI and APO-B_{100} concentrations in children and adolescences, these studies have not examined other APO concentrations (i.e., AII, CII, CIII, E). The information derived from this literature review substantiates the need for more research on the APO variables (i.e., AI, AII, B_{100}, CII, CIII, E) in children and adolescents and their potential association with subclinical atherosclerosis is warranted. The purpose of this study is to examine the association of APOs (i.e., AI, AII, B_{100}, CII, CIII, E) with measures of vascular structure and function (i.e., cIMT, cCSC, cDC, cCSD, cDD, cIEM, PWV) among children and adolescents. We hypothesize that APO concentrations will have a significant correlation with many subclinical cardiovascular disease risk factors.

CHAPTER 3: METHODS

Subject Population

This cross-sectional study included 338 children and adolescents (160 male/178 female) aged 8 to 17 years from the Minneapolis and St. Paul metropolitan area. Participants were recruited from various pediatric clinics, including the University of Minnesota Masonic Children's Hospital Pediatric Weight Management Clinic (participants with obesity or severe obesity only) between 2011-2016. Exclusion from study participation included untreated obstructive sleep apnea, obesity due to a genetic cause, previous medical history of weight loss surgery, current use of antihypertensive medications, type 1 diabetes mellitus, history of hypercholesterolemia, chronic kidney disease or end-stage renal disease, Kawasaki disease, autoimmune inflammatory diseases, and congenital heart disease. Parents and participants provided informed consent and assent, respectively. Study approval was given by the University of Minnesota Institutional Review Board.

Anthropometric Measurements and Pubertal Maturation

Testing was conducted at the University of Minnesota. Height was measured using a wall-stadiometer and weight was measured using a calibrated electronic scale. BMI was calculated by using body mass in kilograms (kg) divided by height in squared meters (m^2). Body composition was measured using standard procedures in the total-body supine position using dual X-ray absorptiometry (iDXA; General Electronic Medical Systems, Madison, WI, USA) and data were analyzed using enCore software version (platform version 16.0, General Electric Medical Systems, Madison, WI). Age and race were self-reported; race was categorized as non-Hispanic White, Latino/Hispanic, Black,

or other. Sex and pubertal stage were determined by a pediatrician or trained nurse using standard Tanner staging procedures (Tanner & Whitehouse, 1976).

Measurement of Lipids and Apolipoprotein Levels

Fasting blood samples (>10 hours) were collected and lipids (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, and insulin) were measured using standard methods in the Fairview Diagnostics Laboratories, Fairview-University Medical Center (Minneapolis, MN, USA), a Center for Disease Control and Prevention certified laboratory. In addition, APO variables (i.e., AI, AII, B_{100}, CII, CIII, and E) were measured with the MILLIPLEX MAP Human Apolipoprotein Magnetic Bead Panel kit (Millipore, MA, USA).

Ultrasound-derived Measures of Arterial Structure and Elasticity

Images of the left carotid artery were obtained via ultrasound (Acuson, Seuoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with an 8.0-15.0 MHz linear array probe. Following 15 minutes of supine rest, luminal systolic and diastolic diameters of the imaged artery were obtained in the longitudinal plane and at a fixed point. The left carotid artery was imaged approximately 1 centimeter proximal from the carotid bulb. Images were collected at 20 frames per second for 10 seconds, concurrently with the measurement of peripheral systolic blood pressure, diastolic blood pressure, and cIMT. All artery images were analyzed using an off-line, electronic wall-tracking software program (Vascular Research Tools 6, Medical Imaging Application, LLC, Iowa city, IA, USA). Mean diameter through the recording was used to calculate both distensibility and compliance. The following calculations were used for arterial

cross-sectional compliance (CSC), diameter compliance (DC), cross-sectional distensibility (CSD), diameter distensibility (DD), and IEM:

$$Cross-sectional\ compliance = \frac{\pi(maxDiamM/2)^2 - \pi(minDiamM/2)^2}{\pi(minDiamM/2)^2 \Delta P}$$

$$Diameter\ compliance = (\frac{maxDiamM - minDiamM}{\Delta P})$$

$$Cross-sectional\ distensibility = \left(\frac{\pi(maxDiamM/2)^2 - \pi(minDiamM/2)^2}{\pi(minDiamM/2)^2}\right) \times 100\%$$

$$Diameter\ distensibility = \left(\frac{maxDiamM - minDiamM}{minDiamM}\right) \times 100\%$$

$$Incremential\ elastic\ modulus = \frac{3 \times [1 + \frac{\pi(minDiamI/2)^2}{\pi(minDiamM/2)^2}]}{cCSC}$$

Where *minDiamM* is the minimum (diastolic) arterial diameter, *maxDiamM* is the maximum (systolic) arterial diameter, *minDiamI* is the minimum lumen diameter, and pulse pressure (ΔP) is calculated as the difference between systolic and diastolic blood pressure (Aggoun et al., 2000; Laurent et al., 2006; Marlatt et al., 2013).

Measurement of Arterial Stiffness

Pulse waveforms were obtained using an arterial tonometer placed over the strongest radial and carotid pulse points relative to the sternal notch. These waveforms were timed to ventricular depolarization via a three-lead ECG. Calculations were made within the tonometer device software (SphygmoCor™, AtCor Medical, Sydney, Australia).

Statistical Analysis

Descriptive statistics were calculated for the participants. Linear regression models assessed the association of each APO variable with measures of vascular structure and function (i.e., cIMT, cCSC, cDC, cCSD, cDD, cIEM, and PWV). Linear regression models were adjusted for Tanner stage, sex, and race with further adjustments for body fat percentage. Data are presented as mean [95% CI] with Holm's-adjusted p-values to account for multiple testing. All APO ratios were scaled to 0.1 μg/mL.

CHAPTER 4: RESULTS

A total of 338 participants (160 males, 178 females) were examined in this study (Table 1). The average age for the participants was 13.0±2.77 years and included individuals from Tanner stage I-V. In addition, while the mean height was 157±14.1 cm and the mean weight was 68.6±29.9 kg, there was a wide range of BMIs. Participants were categorized into four BMI groups: Normal Weight (n=132), Overweight (n=24), Obesity (n=69), and Severe Obesity (n=113). Race was self-reported and included the following groups: African American or Black (n=34), White (n=260), Latino/Hispanic (n=38), and Other (n=44).

The associations of cIMT, cCSC, cDC, cCSD, cDD, cIEM, and PWV with each APO (AI, AII, B_{100}, CII, CIII, E, and the ratio of B_{100}:AI and CIII:CII) adjusted for Tanner stage, sex, and race are displayed in Table 2-8, and is denoted as model 1. Prior to post-hoc adjustment, statically significant, inverse association between cIMT and APO-CII (-0.003 m/sec per 10 μg/mL [-0.005, 0], p=0.02), and a positive association between cIMT and APO-CIII:CII ratio (0.001 [0, 0.003], p=0.033) was observed. However, both associations failed to maintain statistical significance after p-value correction accounting for multiple testing. Prior to post-hoc adjustment there were statistically significant, positive association between PWV and APOs: AI (0.02 m/sec per 10 μg/mL [0.01, 0.03], p=0.003), AII (0.04 m/sec per 10 μg/mL [0.02, 0.06], p<0.001), B_{100} (0.01 m/sec per 10 μg/mL [0.002, 0.02], p=0.012), E (0.16 m/sec per 10 μg/mL [0.08, 0.24], p<0.001), and CIII:CII ratio (0.03 [0.01, 0.05], p<0.001). After adjusting for multiple testing, PWV was statistically significantly associated with APO-AII (0.04 m/s per 10 μg/mL [0.02, 0.06] p=0.046) and APO-E (0.16 m/s per 10 μg/mL [0.08, 0.24], p=0.012). There were no

statistically significant associations between any of the apolipoproteins and cIMT, cCSC, cDC, cCSD, cDD, and cIEM.

We conducted further multiple linear regression analysis to account for any effect of body fatness (body fat percent) (Tables 2-8, model 2). Following adjustment for body fat percent, PWV remained significantly, positively associated with higher levels of APO-E (0.16 m/sec per 10 μg/mL [0.08, 0.24], p=0.01). There were no significant associations between any APO and cIMT, cCSC, cDC, cCSD, cDD, or cIEM.

CHAPTER 5: DISCUSSION

To the best of our knowledge, the current study is the first to examine the association of multiple APOs to measures of vascular structure and function in a pediatric population. The most important study observations included statistically significant associations in APO-E with PWV, both with and without the adjustment of percent body fat. Surprisingly, although there were associations in APOs and PWV, there were no significant associations with cIMT, cCSC, cDC, cCSD, cDD, or cIEM. Taken together, results from the present study indicate that APOs, specifically APO-E, may exert its effects on vascular function before altering vascular structure, though longitudinal studies assessing the chronology will be needed to confirm this observation.

Previous literature on the association of the majority of APO variables and cardiovascular disease risk factors have primarily been in adults (Gerber et al., 2002; Huang et al., 2013; Kei et al., 2012; Ooi et al., 2008; Sacks et al., 2000; Schaefer et al., 1994; Sniderman et al., 2007, 2016; Steffen et al., 2017; Stewart et al., 2007; Zivanovic et al., 2018). Notably, these studies have reported that decreased concentrations of APO-AI, APO-AII, and APO-E and increased concentrations of APO-$B_{100,}$ APO-B_{100}:AI ratio, and APO-CII may play a role in the risk of cardiovascular disease (Kappelle et al., 2011; Kei et al., 2012; McQueen et al., 2008; Pischon et al., 2005; Sharrett et al., 1994; Sniderman et al., 2007, 2016; Stewart et al., 2007). However, previous literature in children have predominately focused on concentrations of APO-AI and APO-B_{100}. This could be due to the concentration of APO-AI represents the number of proatherogenic lipoproteins. Whereas, the concentrations of APO-B_{100} is a direct measure of the number of atherogenic lipoproteins. Specifically, these studies conducted have explored the

relationship between APO concentrations in children of parents who had either early onset coronary heart disease, history of cardiovascular disease, or myocardial infarction. These studies reported children of patients had lower concentrations of APO-AI and APO-AII, and higher APO-B_{100} and APO-B_{100}:AI ratio (Beigel et al., 1993; Bornaun et al., 2017; Freedman et al., 1986; Uiterwaal et al., 1996).

In addition, previous studies in children have explored APO-AI and APO-B_{100} concentrations across BMI (Hobkirk et al., 2013; Krekoukia et al., 2007; Teixeira et al., 2001). These studies report associations of APO-AI and APO-B_{100} with adiposity. Specifically, lower levels of APO-AI and higher levels of APO-B_{100} in children with obesity compared to their counterparts without obesity have been reported (Krekoukia et al., 2007; Teixeira et al., 2001). This is important since childhood obesity affects vascular structure and function and ultimately may increase the risk of cardiovascular disease (Dengel & Bronas, 2010). Therefore, the current understanding from the literature is that APOs may have minimal impact during childhood and adolescence but can predispose such individuals to substantial increased risk of cardiovascular disease in adulthood.

To date, few studies conducted in adults and children have explored the association between APOs and measures of vascular structure and function. In adults, patients with increased cIMT had higher mean APO-B_{100} and APO-B_{100}:AI ratio and lower APO-AI, with the most significant predictor of increased cIMT being the APO-B_{100}:AI ratio (Sharrett et al., 1994; Wallenfeldt et al., 2004; Zivanovic et al., 2018). Similarly, in children, studies reported that adulthood cIMT was inversely related with APO-AI and positively associated with APO-B_{100} and APO-B_{100}:AI ratio (Frontini et al., 2008; Juonala et al., 2008; Kallio et al., 2010). However, two of these studies were part of

long-term cohort studies that started in childhood, but the researchers obtained ultrasound measures of cIMT in adulthood (Frontini et al., 2008; Juonala et al., 2008). The current study explores the relationship of cIMT and a variety of APO variables, not just APO-AI and APO-B_{100}. Interestingly, our study found no association between cIMT and any of the APO variables after post-hoc adjustment. These differences between study results could be influenced by the heterogeneity of the population and differences in geographic location. Therefore, the differences in cohorts and the effects of time may be the reason the present study's observations were different. Also, APOs may have either a small impact on childhood cIMT or the effects on cIMT are not present yet at an early stage of disease.

The effect of APOs on vascular health has predominately been restricted to analysis of flow-mediated dilation. These studies examining APO concentrations in relation to flow-mediated dilation have reported a strong positive correlation between flow-mediated dilation and APO-AI concentration in children with obesity, and a direct relationship between APO-AI and adulthood flow-mediated dilation (Juonala et al., 2008; Tounian et al., 2001). These results indicate a possible relationship between low APO-AI levels and endothelial dysfunction in children and the potential future effects of APO concentrations. While flow-mediated dilation is a measure of vascular function, specifically endothelial dysfunction of the brachial artery, this measure was not used in the present study. Instead, the present study assessed vascular function via applanation tonometry. Through this measurement multiple arteries can be evaluated (i.e., carotid, radial, femoral). However, in the current study we assessed the carotid and radial artery, and observed a significant positive association with PWV and APO-AII and APO-E.

Also, after further adjustment for body fat percent, a significant positive association with PWV and APO-E. These data may indicate these specific APOs may impact vascular function early in life. Notably, the opposing roles of APO-AII in lipoprotein metabolism (i.e., increased lecithin-cholesterol acyltransferase, decreased hepatic lipase and cholesteryl ester transfer protein) provide conflicting evidence in regard to cardiovascular disease risk. In addition, APO-E regulates the metabolism of lipoprotein containing APO-B. Therefore, depending on the concentration of APO-E and the specific genotype, there may be more low-density lipoprotein cholesterol. Ultimately impacting arterial stiffness. Therefore, the complex roles of APO-AII and APO-E may explain why the present study's observations may be different than previous studies.

While the current study evaluated the effect of APO variables, and not age or body fat, on arterial structure and stiffness, we report that changes indicative of increased arteriosclerosis risk occurs first with measures of arterial stiffness. Specifically, PWV was impacted prior to other measures of vascular function (i.e., cCSC, cDC, cCSD, cDD, cIEM). However, this may be due to the differences in measures. The ultrasound derived measures were used for the carotid artery. While, applanation tonometry measures were from two sites, the radial and carotid artery. Previous studies have reported that signs of atherosclerosis occur at multiple vascular beds and the development of lesions at each bed is dependent on a variety of factors (i.e., genetic background, gender, oxidative stress) (Frangos, Gahtan, & Sumpio, 1999; VanderLaan, Reardon, & Getz, 2004). In addition, ultrasound derived measures provide images of the carotid artery as two dimensional and is a measure of regional vascular function. Whereas, applanation tonometry uses the sensor in the tonometer and pressure of the artery to provide

information on the pulse waveform. Therefore, PWV uses waveform travel between two sites and provides a measure of systemic arterial stiffness (Nelson et al., 2010; O'Rourke et al., 2002). Hence, the differences in measures may factor into why there were associations with PWV and not the ultrasound derived measures of vascular function.

Strengths of this study include the relatively large sample size, inclusion of participants with a wide range of BMI values, the use of dual X-ray absorptiometry for measures of body composition, and the use of standardized methods used for measuring subclinical vascular structure and function. Limitations include the cross-sectional nature of the study, which prevents us from suggesting causality between APO concentrations and either increased or decreased cIMT, cCSC, cDC, cCSD, cDD, cIEM, or PWV or potential changes in these associations over time. It is also important to note that neither categories nor percentiles exist for APO concentrations, which limits the ability to interpret the findings from a clinical perspective.

CHAPTER 6: CONCLUSION

In summary, these findings suggest higher levels of APO-E are associated with higher systemic arterial stiffness in children and adolescents, both with and without the adjustment for body fat percent. This study reported that APO-E may exert its effects on vascular function, specifically arterial stiffness, before altering vascular structure. However, further investigation of APOs as biomarkers for vascular structure in pediatrics is warranted. Also, future research is needed to assess how APO concentrations change over the childhood and adolescence period and the potential impact of body fat or pubertal maturation on those changes.

Table 1. Cohort demographics and anthropometrics

	Overall	Male	Female
N	338	160	178
Race/Ethnicity:			
-African American or Black	34 (10.1%)	14 (8.8%)	20 (11.2%)
-White	260 (76.9%)	123 (76.9%)	137 (77.0%)
-Other	44 (13.0%)	23 (14.4%)	21 (11.8%)
- Latino/Hispanic	38 (11.2%)	20 (12.5%)	18 (10.1%)
Tanner Stage:			
-Stage I	85 (25.1%)	51 (31.9%)	34 (19.1%)
-Stage II/III/IV	182 (53.8%)	78 (48.8%)	104 (58.4%)
-Stage V	57 (16.9%)	24 (15.0%)	33 (18.5%)
Obesity Status:			
-Normal	132 (39.1%)	75 (46.9%)	57 (32.0%)
-Overweight	24 (7.1%)	10 (6.2%)	14 (7.9%)
-Obesity	69 (20.4%)	32 (20.0%)	37 (20.8%)
-Severe Obesity	113 (33.4%)	43 (26.9%)	70 (39.3%)
Age (years)	13.0 (2.77)	13.0 (2.52)	13.0 (2.99)
Weight (kg)	68.6 (29.9)	67.6 (32.0)	69.5 (27.9)
Height (cm)	157 (14.1)	160 (15.5)	155 (12.4)
BMI (kg/m^2)	26.8 (8.92)	25.4 (8.8)	28.0 (8.87)
BMI Percentile (%)	78.2 (27.8)	74.7 (28.4)	81.4 (27.0)
Body Fat Percent (%)	36.8 (11.7)	32.5 (12.2)	40.7 (9.72)

Continuous variables are presented as mean +/- standard deviation (SD).
Categorical variables (e.g., race, Tanner stage, obesity status) are presented as count (% within column).
Obesity status is presented as normal weight, overweight, obesity, severe obesity
All who are missing BMI or any apolipoprotein measure were excluded
Abbreviations: BMI = body mass index

Table 2. Associations of apolipoprotein variables with measures of vascular structure

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cIMT (mm)						
APO AI	0 (0, 0.001)	0.535	>0.999	0 (-0.001, 0.001)	0.728	>0.999
APO AII	0.001 (0, 0.002)	0.172	>0.999	0.001 (0, 0.002)	0.194	>0.999
APO B_{100}	0 (-0.001, 0)	0.454	>0.999	0 (0, 0)	0.908	>0.999
APO CII	-0.003 (-0.005, 0)	0.02	0.924	-0.002 (-0.004, 0)	0.059	>0.999
APO CIII	-0.001 (-0.002, 0.001)	0.326	>0.999	0 (-0.001, 0.001)	0.548	>0.999
APO E	0.001 (-0.004, 0.006)	0.663	>0.999	0.003 (-0.003, 0.008)	0.309	>0.999
APO B_{100}:AI	0 (-0.001, 0.001)	0.936	>0.999	0 (0, 0.001)	0.362	>0.999
APO CIII:CII	0.001 (0, 0.003)	0.033	>0.999	0.001 (0, 0.003)	0.049	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cIMT = carotid intima-media thickness

Table 3. Associations of apolipoprotein variables with measures of carotid cross-sectional compliance

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cCSC (1/mmHg x 10^{-3})						
APO AI	-0.01 (-0.02, 0.01)	0.366	>0.999	-0.1 (-0.03, -0.002)	0.086	>0.999
APO AII	-0.02 (-0.05, 0.02)	0.326	>0.999	-0.02 (-0.05, 0.01)	0.268	>0.999
APO B_{100}	-0.01 (-0.02, 0.004)	0.186	>0.999	-0.004 (-0.02, 0.01)	0.454	>0.999
APO CII	-0.04 (-0.09, 0.02)	0.202	>0.999	-0.03 (-0.08, 0.03)	0.343	>0.999
APO CIII	-0.02 (-0.05, 0.004)	0.089	>0.999	-0.02 (-0.05, 0.01)	0.149	>0.999
APO E	-0.04 (-0.17, 0.08)	0.501	>0.999	-0.02 (-0.14, 0.11)	0.765	>0.999
APO B_{100}:AI	-0.003 (-0.02, 0.01)	0.704	>0.999	0.003 (-0.01, 0.02)	0.658	>0.999
APO CIII:CII	-0.01 (-0.04, 0.02)	0.407	>0.999	-0.01 (-0.04, 0.02)	0.352	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cCSC = carotid cross-sectional compliance

Table 4. Associations of apolipoprotein variables with measures of carotid diameter compliance

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cDC (mm/mmHg x 10^{-3})						
APO AI	-0.03 (-0.07, 0.02)	0.208	>0.999	-0.04 (-0.08, -0.001)	0.044	>0.999
APO AII	-0.04 (-0.12, 0.03)	0.279	>0.999	-0.05 (-0.13, 0.03)	0.228	>0.999
APO B_{100}	-0.02 (-0.05, 0.003)	0.076	>0.999	-0.02 (-0.05, 0.01)	0.206	>0.999
APO CII	-0.07 (-0.21, 0.07)	0.319	>0.999	-0.05 (-0.19, 0.09)	0.489	>0.999
APO CIII	-0.05 (-0.12, 0.02)	0.165	>0.999	-0.04 (-0.11, 0.03)	0.25	>0.999
APO E	-0.10 (-0.41, 0.21)	0.52	>0.999	-0.05 (-0.36, 0.26)	0.753	>0.999
APO B_{100}:AI	-0.003 (-0.04, 0.03)	0.87	>0.999	0.01 (-0.03, 0.05)	0.549	>0.999
APO CIII:CII	-0.03 (-0.1, 0.05)	0.52	>0.999	-0.03 (-0.10, 0.05)	0.461	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cDC = carotid diameter compliance

Table 5. Associations of apolipoprotein variables with measures of carotid cross-sectional distensibility

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cCSD (%)						
APO AI	-0.05 (-0.11, 0.01)	0.084	>0.999	-0.06 (-0.12, 0.01)	0.055	>0.999
APO AII	-0.06 (-0.17, 0.05)	0.288	>0.999	-0.06 (-0.17, 0.05)	0.272	>0.999
APO B_{100}	-0.02 (-0.06, 0.02)	0.387	>0.999	-0.02 (-0.06, 0.02)	0.441	>0.999
APO CII	-0.07 (-0.27, 0.13)	0.481	>0.999	-0.07 (-0.26, 0.13)	0.52	>0.999
APO CIII	-0.04 (-0.14, 0.05)	0.384	>0.999	-0.04 (-0.14, 0.06)	0.409	>0.999
APO E	0.06 (-0.39, 0.50)	0.807	>0.999	0.07 (-0.38, 0.51)	0.773	>0.999
APO B_{100}:AI	0.03 (-0.02, 0.09)	0.207	>0.999	0.04 (-0.01, 0.10)	0.145	>0.999
APO CIII:CII	-0.04 (-0.14, 0.07)	0.512	>0.999	-0.04 (-0.14, 0.07)	0.499	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cCSD = carotid cross-sectional distensibility

Table 6. Associations of apolipoprotein variables with measures of carotid diameter distensibility

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cDD (%)						
APO AI	-0.02 (-0.04, 0.01)	0.172	>0.999	-0.02 (-0.05, 0.01)	0.123	>0.999
APO AII	-0.02 (-0.07, 0.03)	0.386	>0.999	-0.02 (-0.07, 0.03)	0.366	>0.999
APO B_{100}	-0.004 (-0.02, 0.01)	0.63	>0.999	-0.003 (-0.02, 0.01)	0.703	>0.999
APO CII	-0.02 (-0.11, 0.06)	0.615	>0.999	-0.02 (-0.10, 0.07)	0.659	>0.999
APO CIII	-0.01 (-0.05, 0.03)	0.572	>0.999	-0.01 (-0.05, 0.03)	0.603	>0.999
APO E	0.01 (-0.18, 0.20)	0.916	>0.999	0.01 (-0.18, 0.21)	0.883	>0.999
APO B_{100}:AI	0.01 (-0.01, 0.04)	0.244	>0.999	0.02 (-0.01, 0.04)	0.175	>0.999
APO CIII:CII	-0.01 (-0.06, 0.03)	0.582	>0.999	-0.01 (-0.04, 0.02)	0.568	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cDD = carotid diameter distensibility

Table 7. Associations of apolipoprotein variables with measures of carotid incremental elastic modulus

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
cIEM (mmHg)						
APO AI	-0.57 (-3.26, 2.11)	0.674	>0.999	0.95 (-1.70, 3.61)	0.481	>0.999
APO AII	-0.17 (-5.33, 4.99)	0.948	>0.999	0.22 (-4.76, 5.19)	0.932	>0.999
APO B_{100}	1.24 (-0.56, 3.04)	0.176	>0.999	0.45 (-1.32, 2.22)	0.615	>0.999
APO CII	4.27 (-4.83, 13.37)	0.356	>0.999	1.94 (-6.89, 10.77)	0.666	>0.999
APO CIII	2.57 (-1.86, 6.99)	0.255	>0.999	1.63 (-2.66, 5.91)	0.456	>0.999
APO E	-0.68 (-21.22, 19.87)	0.948	>0.999	-6.82 (-26.81, 13.17)	0.503	>0.999
APO B_{100}:AI	1.09 (-1.38, 3.56)	0.385	>0.999	-0.42 (-2.87, 2.04)	0.739	>0.999
APO CIII:CII	0.69 (-4.27, 5.64)	0.785	>0.999	1.01 (-3.77, 5.79)	0.678	>0.999

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: cIEM = carotid incremental elastic modulus

Table 8. Associations of apolipoprotein variables with measures of vascular stiffness

	Model 1			Model 2		
Covariate	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**	**Difference (95%CI)**	**Unadjusted P-value**	**Adjusted P-value**
Radial-carotid PWV (m/s)						
APO AI	0.02 (0.01, 0.03)	0.003	0.463	0.02 (0.01, 0.03)	0.004	0.516
APO AII	0.04 (0.02, 0.06)	<0.001	0.046	0.04 (0.02, 0.06)	<0.001	0.054
APO B_{100}	0.01 (0.002, 0.02)	0.012	>0.999	0.01 (0.003, 0.02)	0.008	>0.999
APO CII	0 (-0.04, 0.04)	0.986	>0.999	0.001 (-0.03, 0.04)	0.943	>0.999
APO CIII	0.02 (-0.002, 0.03)	0.078	>0.999	0.02 (-0.001, 0.03)	0.072	>0.999
APO E	0.16 (0.08, 0.24)	<0.001	0.012	0.16 (0.08, 0.24)	<0.001	0.010
APO B_{100}:AI	-0.01 (-0.02, 0.001)	0.067	>0.999	-0.01 (-0.02, 0.001)	0.078	>0.999
APO CIII:CII	0.03 (0.01, 0.05)	<0.001	0.085	0.03 (0.01, 0.05)	<0.001	0.091

Model 1 is adjusted for Tanner stage, sex, and race; Model 2 further adjusts Model 1 for body fat percent. Adjusted p-values were calculated using Holm's method.

Data presented are mean difference and 95% confidence interval estimates for each apolipoprotein. Additionally, apolipoproteins are scaled to 10 μg/mL and ratios were scaled to 0.1 μg/mL.

Abbreviations: PWV = Pulse wave velocity

REFERENCES

Aggoun, Y., Sidi, D., Levy, B. I., Lyonnet, S., Kachaner, J., & Bonnet, D. (2000). Mechanical properties of the common carotid artery in Williams syndrome. *Heart*, *84*(3), 290–293. https://doi.org/10.1136/heart.84.3.290

Alemzadeh, R., & Kichler, J. (2018). Comparison of Apolipoprotein (ApoB/ApoA-1) and Lipoprotein (Total Cholesterol/HDL) Ratios in Obese Adolescents. *Metabolic Syndrome and Related Disorders*, *16*(1), 40–45. https://doi.org/10.1089/met.2017.0135

Anagnostis, P., Stevenson, J. C., Crook, D., Johnston, D. G., & Godsland, I. F. (2016). Effects of gender, age and menopausal status on serum apolipoprotein concentrations. *Clinical Endocrinology*, *85*(5), 733–740. https://doi.org/10.1111/cen.13085

Beigel, Y., George, J., Leibovici, L., Mattityahu, A., Sclarovsky, S., & Blieden, L. (1993). Coronary risk factors in children of parents with premature coronary artery disease. *Acta Pædiatrica*, *82*(2), 162–165. https://doi.org/10.1111/j.1651-2227.1993.tb12631.x

Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P., Tracy, R. E., & Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. *New England Journal of Medicine*, *338*(23), 1650–1656. https://doi.org/10.1056/NEJM199806043382302

Bornaun, H., Öner, N., Nisli, K., Öztarhan, K., Yavuz, T., Türkoglu, Ü., … Ömeroglu, R. E. (2017). Assessment of lipid profile and some risk factors of atherosclerosis in children whose parents had early onset coronary artery disease. *Archivos Argentinos de Pediatria*, *115*(1), 50–53. https://doi.org/10.5546/aap.2017.eng.50

Burke, G. L., Bertoni, A. G., Shea, S., Tracy, R., Watson, K. E., Blumenthal, R. S., … Carnethon, M. R. (2008). The impact of obesity on cardiovascular disease risk factors and subclinical vascular disease: The multi-ethnic study of atherosclerosis. *Archives of Internal Medicine*, *168*(9), 928–935. https://doi.org/10.1001/archinte.168.9.928

Davignon, J., Gregg, R. E., & Sing, C. F. (1988). Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis*, *8*(1), 1–21. https://doi.org/10.1161/01.atv.8.1.1

Dengel, D. R., & Bronas, U. G. (2010). The Role of Endothelial Dysfunction on Development and Progression of Atherosclerosis and Methods to Assess Vascular Function and Structure. *American Journal of Lifestyle Medicine*, *4*(5), 445–456.

Doliner, B., Dong, C., Blanton, S. H., Gardener, H., Elkind, M. S. V., Sacco, R. L., … Rundek, T. (2018). Apolipoprotein E Gene Polymorphism and Subclinical Carotid Atherosclerosis: The Northern Manhattan Study. *Journal of Stroke and Cerebrovascular Diseases*, *27*(3), 645–652. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.09.053

Donma, M. M., & Donma, O. (1989). APOLIPOPROTEINS : BIOCHEMISTRY , METHODS AND CLINICAL. *Biochemical Education*, *17*(2), 63–68.

Erdeve, S. S., Simsek, E., Dallar, Y., & Biyikli, Z. (2010). Utility of apoB/apoA1 ratio for the prediction of cardiovascular risk in childrenwith metabolic syndrome. *Indian Journal of Pediatrics*, *77*(11), 1261–1265. https://doi.org/10.1007/s12098-010-0217-8

Frangos, S. G., Gahtan, V., & Sumpio, B. (1999). Localization of atherosclerosis: Role of hemodynamics. *Archives of Surgery*, *134*(10), 1142–1149. https://doi.org/10.1001/archsurg.134.10.1142

Frontini, M. G., Srinivasan, S. R., Xu, J., Tang, R., Bond, M. G., & Berenson, G. S. (2008). Usefulness of childhood non-high density lipoprotein cholesterol levels versus other lipoprotein measures in predicting adult subclinical atherosclerosis: The bogalusa heart study. *Pediatrics*, *121*(5), 924–929. https://doi.org/10.1542/peds.2007-1472

Gerber, Y., Goldbourt, U., Cohen, H., & Harats, D. (2002). Association between serum apolipoprotein CII concentration and coronary heart disease. *Preventive Medicine*, *35*(1), 42–47. https://doi.org/10.1006/pmed.2002.1045

Hobkirk, J. P., King, R. F., Davies, I., Harman, N., Gately, P., Pemberton, P., … Carroll, S. (2014). The metabolic inter-relationships between changes in waist circumference, triglycerides, insulin sensitivity and small, dense low-density lipoprotein particles with acute weight loss in clinically obese children and adolescents. *Pediatric Obesity*, Vol. 9, pp. 209–217. https://doi.org/10.1111/j.2047-6310.2013.00165.x

Huang, F., Yang, Z., Xu, B., Bi, Y., Xu, M., Xu, Y., … Chen, Y. (2013). Both Serum Apolipoprotein B and the Apolipoprotein B/Apolipoprotein A-I Ratio Are Associated with Carotid Intima-Media Thickness. *PLoS ONE*, *8*(1), 1–7. https://doi.org/10.1371/journal.pone.0054628

Jani, B., & Rajkumar, C. (2006). Ageing and vascular ageing. *Postgraduate Medical Journal*, *82*(968), 357–362. https://doi.org/10.1136/pgmj.2005.036053

Juonala, M., Viikari, J. S. A., Kähönen, M., Solakivi, T., Helenius, H., Jula, A., … Raitakari, O. T. (2008). Childhood Levels of Serum Apolipoproteins B and A-I Predict Carotid Intima-Media Thickness and Brachial Endothelial Function in Adulthood. The Cardiovascular Risk in Young Finns Study. *Journal of the American College of Cardiology*, *52*(4), 293–299. https://doi.org/10.1016/j.jacc.2008.03.054

Kallio, K., Jokinen, E., Saarinen, M., Hämäläinen, M., Volanen, I., Kaitosaari, T., … Simell, O. (2010). Arterial intima-media thickness, endothelial function, and apolipoproteins in adolescents frequently exposed to tobacco smoke. *Circulation: Cardiovascular Quality and Outcomes*, *3*(2), 196–203. https://doi.org/10.1161/CIRCOUTCOMES.109.857771

Kappelle, P. J. W. H., Gansevoort, R. T., Hillege, J. L., Wolffenbuttel, B. H. R., & Dullaart, R. P. F. (2011). Apolipoprotein B/A-I and total cholesterol/high-density lipoprotein cholesterol ratios both predict cardiovascular events in the general population independently of nonlipid risk factors, albuminuria and C-reactive protein. *Journal of Internal Medicine*, *269*(2), 232–242. https://doi.org/10.1111/j.1365-2796.2010.02323.x

Karabouta, Z., Papandreou, D., Makedou, A., Rousso, I., & Athanassiadou, F. (2016). Associations of Apolipoprotein A, High-Sensitivity C-Reactive Protein and Fasting Plasma Insulin in Obese Children With and Without Family History of Cardiovascular Disease. *Journal of Clinical Medicine Research*, *8*(6), 431–436. https://doi.org/10.14740/jocmr2531w

Kei, A. A., Filippatos, T. D., Tsimihodimos, V., & Elisaf, M. S. (2012). A review of the

role of apolipoprotein C-II in lipoprotein metabolism and cardiovascular disease. *Metabolism: Clinical and Experimental*, *61*(7), 906–921. https://doi.org/10.1016/j.metabol.2011.12.002

Kolovou, G., Daskalova, D., & Mikhailidis, D. P. (2003). Apolipoprotein E Polymorphism and Atherosclerosis. *Angiology*, *54*(1), 59–71. https://doi.org/10.1053/srin.2003.50006

Kotsis, V. T., Stabouli, S. V., Papamichael, C. M., & Zakopoulos, N. A. (2006). Impact of obesity in intima media thickness of carotid arteries. *Obesity*, *14*(10), 1708–1715. https://doi.org/10.1038/oby.2006.196

Krekoukia, M., Nassis, G. P., Psarra, G., Skenderi, K., Chrousos, G. P., & Sidossis, L. S. (2007). Elevated total and central adiposity and low physical activity are associated with insulin resistance in children. *Metabolism: Clinical and Experimental*, *56*(2), 206–213. https://doi.org/10.1016/j.metabol.2006.09.014

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., … Struijker-Boudier, H. (2006). Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *European Heart Journal*, *27*(21), 2588–2605. https://doi.org/10.1093/eurheartj/ehl254

Lloyd-Jones, D. M., Leip, E. P., Larson, M. G., D'Agostino, R. B., Beiser, A., Wilson, P. W. F., … Levy, D. (2006). Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation*, *113*(6), 791–798. https://doi.org/10.1161/CIRCULATIONAHA.105.548206

Marlatt, K. L., Kelly, A. S., Steinberger, J., & Dengel, D. R. (2013). The influence of gender on carotid artery compliance and distensibility in children and adults. *Journal of Clinical Ultrasound*, *41*(6), 340–346. https://doi.org/10.1002/jcu.22015

McQueen, M. J., Hawken, S., Wang, X., Ounpuu, S., Sniderman, A., Probstfield, J., … Yusuf, S. (2008). Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *The Lancet*, *372*(9634), 224–233. https://doi.org/10.1016/S0140-6736(08)61076-4

Nelson, M. R., Stepanek, J., Cevette, M., Covalciuc, M., Hurst, R. T., & Tajik, A. J. (2010). Noninvasive measurement of central vascular pressures with arterial tonometry: Clinical revival of the pulse pressure waveform? *Mayo Clinic Proceedings*, *85*(5), 460–472. https://doi.org/10.4065/mcp.2009.0336

O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C., Duprez, D., & Plante, G. E. (2002). Clinical applications of arterial stiffness; definitions and reference values. *American Journal of Hypertension*, *15*(5), 426–444. https://doi.org/10.1016/S0895-7061(01)02319-6

Ooi, E. M. M., Barrett, P. H. R., Chan, D. C., & Watts, G. F. (2008). Apolipoprotein C-III: Understanding an emerging cardiovascular risk factor. *Clinical Science*, *114*(9–10), 611–624. https://doi.org/10.1042/CS20070308

Pischon, T., Girman, C. J., Sacks, F. M., Rifai, N., Stampfer, M. J., & Rimm, E. B. (2005). Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation*, *112*(22), 3375–3383. https://doi.org/10.1161/CIRCULATIONAHA.104.532499

Rader, D. J., Hoeg, J. M., & Brewer, H. B. (1994). Quantitation of plasma apolipoproteins in the primary and secondary prevention of coronary artery disease.

Annals of Internal Medicine, *120*(12), 1012–1025. https://doi.org/10.7326/0003-4819-120-12-199406150-00008

Rainwater, D. L., McMahan, C. A., Malcom, G. T., Scheer, W. D., Roheim, P. S., McGill, H. C., & Strong, J. P. (1999). Lipid and apolipoprotein predictors of atherosclerosis in youth. Apolipoprotein concentrations do not materially improve prediction of arterial lesions in PDAY subjects. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *19*(3), 753–761. https://doi.org/10.1161/01.ATV.19.3.753

Reneman, R. S., Meinders, J. M., & Hoeks, A. P. G. (2005). Non-invasive ultrasound in arterial wall dynamics in humans: What have we learned and what remains to be solved. *European Heart Journal*, *26*(10), 960–966. https://doi.org/10.1093/eurheartj/ehi177

Sacks, F. M., Alaupovic, P., Moye, L. a, Cole, T. G., Sussex, B., Stampfer, M. J., … Braunwald, E. (2000). Recurrent Coronary Events in the Cholesterol and. *Circulation*, *102*, 1886–1892.

Sakurabayashi, I., Saito, Y., Kita, T., Matsuzawa, Y., & Goto, Y. (2001). Reference intervals for serum apolipoproteins A-I, A-II, B, C-II, C-III, and E in healthy Japanese determined with a commercial immunoturbidimetric assay and effects of sex, age, smoking, drinking, and Lp(a) level. *Clinica Chimica Acta*, *312*(1–2), 87–95. https://doi.org/10.1016/S0009-8981(01)00591-5

Schaefer, E. J., Lamon-Fava, S., Cohn, S. D., Schaefer, M. M., Ordovas, J. M., Castelli, W. P., & Wilson, P. W. F. (1994). Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *Journal of Lipid Research*, *35*(5), 779–792.

Sharrett, A. R., Patsch, W., Sorlie, P. D., Heiss, G., Bond, M. G., & Davis, C. E. (1994). Associations of lipoprotein cholesterols, apolipoproteins A-I and B, and triglycerides with carotid atherosclerosis and coronary heart disease: The atherosclerosis risk in communities (ARIC) study. *Arteriosclerosis and Thrombosis*, *14*(7), 1098–1104.

Slooter, A., Bots, M., Havekes, L., Iglesias del Sol, A., Crusts, M., Grobbee, D., … van Duijn, C. (2001). Apolipoprotein E and Carotid Artery Atherosclerosis: The Rotterdam Study. *Stroke*, *32*, 1947–1952.

Sniderman, A. D., Holme, I., Aastveit, A., Furberg, C., Walldius, G., & Jungner, I. (2007). Relation of Age, the Apolipoprotein B/Apolipoprotein A-I Ratio, and the Risk of Fatal Myocardial Infarction and Implications for the Primary Prevention of Cardiovascular Disease. *American Journal of Cardiology*, *100*(2), 217–221. https://doi.org/10.1016/j.amjcard.2007.02.086

Sniderman, A. D., Islam, S., McQueen, M., Pencina, M., Furberg, C. D., Thanassoulis, G., & Yusuf, S. (2016). Age and cardiovascular risk attributable to apolipoprotein b, low-density lipoprotein cholesterol or non-high-density lipoprotein cholesterol. *Journal of the American Heart Association*, *5*(10), 7–9. https://doi.org/10.1161/JAHA.116.003665

Song, Y., Stampfer, M. J., & Liu, S. (2004). Meta-Analysis: Apolipoprotein E Genotypes and Risk for Coronary Heart Disease. *Annals of Internal Medicine*, *141*, 137–147.

Steffen, B. T., Guan, W., Remaley, A. T., Stein, J. H., Tattersall, M. C., Kaufman, J., & Tsai, M. Y. (2017). Apolipoprotein B is associated with carotid atherosclerosis progression independent of individual cholesterol measures in a 9-year prospective

study of Multi-Ethnic Study of Atherosclerosis participants. *Journal of Clinical Lipidology*, Vol. 11, pp. 1181-1191.e1. https://doi.org/10.1016/j.jacl.2017.07.001

Steinberg, H. O., Chaker, H., Leaming, R., Johnson, A., Brechtel, G., & Baron, A. D. (1996). Obesity/insulin resistance is associated with endothelial dysfunction: Implications for the syndrome of insulin resistance. *Journal of Clinical Investigation*, *97*(11), 2601–2610. https://doi.org/10.1172/JCI118709

Stewart, C. R., Haw, A., Lopez, R., McDonald, T. O., Callaghan, J. M., McConville, M. J., … O'Brien, K. D. (2007). Serum amyloid P colocalizes with apolipoproteins in human atheroma: Functional implications. *Journal of Lipid Research*, *48*(10), 2162–2171. https://doi.org/10.1194/jlr.M700098-JLR200

Tailleux, A., Duriez, P., Fruchart, J. C., & Clavey, V. (2002). Apolipoprotein A-II, HDL metabolism and atherosclerosis. *Atherosclerosis*, *164*(1), 1–13. https://doi.org/10.1016/S0021-9150(01)00751-1

Teixeira, P. J., Sardinha, L. B., Going, S. B., & Lohman, T. G. (2001). Total and regional fat and serum cardiovascular disease risk factors in lean and obese children and adolescents. *Obesity Research*, *9*(8), 432–442. https://doi.org/10.1038/oby.2001.57

Tian, L., & Fu, M. (2011). The relationship between high density lipoprotein subclass profile and apolipoprotein concentrations. *Journal of Endocrinological Investigation*, *34*(6), 461–472. https://doi.org/10.1007/BF03346714

Tounian, P., Aggoun, Y., Dubern, B., Varille, V., Guy-Grand, B., Sidi, D., … Bonnet, D. (2001). Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: A prospective study. *Lancet*, *358*(9291), 1400–1404. https://doi.org/10.1016/S0140-6736(01)06525-4

Uiterwaal, C. S. P. M., Witteman, J. C. M., Van Stiphout, W. A. H. J., Krauss, X. H., De Bruijn, A. M., Hofman, A., & Grobbee, D. E. (1996). Lipoproteins and apolipoproteins in the young and familial risk of coronary atherosclerosis. *Atherosclerosis*, *122*(2), 235–244. https://doi.org/10.1016/0021-9150(95)05758-7

Utermann, G., Hardewig, A., & Zimmer, F. (1984). Apolipoprotein E phenotypes in patients with myocardial infarction. *Human Genetics*, *65*(3), 237–241. https://doi.org/10.1007/BF00286509

VanderLaan, P. A., Reardon, C. A., & Getz, G. S. (2004). Site Specificity of Atherosclerosis: Site-Selective Responses to Atherosclerotic Modulators. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*(1), 12–22. https://doi.org/10.1161/01.ATV.0000105054.43931.f0

Volcik, K. A., Barkley, R. A., Hutchinson, R. G., Mosley, T. H., Heiss, G., Sharrett, A. R., … Boerwinkle, E. (2006). Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. *American Journal of Epidemiology*, *164*(4), 342–348. https://doi.org/10.1093/aje/kwj202

Walldius, G., & Jungner, I. (2007). Apolipoprotein A-I versus HDL cholesterol in the prediction of risk for myocardial infarction and stroke. *Current Opinion in Cardiology*, *22*(4), 359–367. https://doi.org/10.1097/HCO.0b013e3281bd8849

Wallenfeldt, K., Bokemark, L., Wikstrand, J., Hulthe, J., & Fagerberg, B. (2004). Apolipoprotein B/apolipoprotein A-I in relation to the metabolic syndrome and change in carotid artery intima-media thickness during 3 years in middle-aged men. *Stroke*, *35*(10), 2248–2252. https://doi.org/10.1161/01.STR.0000140629.65145.3c

Williams, I. L., Chowienczyk, P. J., Wheatcroft, S. B., Patel, A. G., Sherwood, R. A., Momin, A., … Kearney, M. T. (2005). Endothelial function and weight loss in obese humans. *Obesity Surgery*, *15*(7), 1055–1060. https://doi.org/10.1381/0960892054621134

Woo, K. S., Chook, P., Yu, C. W., Sung, R. Y. T., Qiao, M., Leung, S. S. F., … Celermajer, D. S. (2004). Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. *International Journal of Obesity*, *28*(7), 852–857. https://doi.org/10.1038/sj.ijo.0802539

Yasue, H., Matsuyama, K., Matsuyama, K., Okumura, K., Morikami, Y., & Ogawa, H. (1990). Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment: Possible role of early coronary atherosclerosis. *Circulation*, *81*(2), 482–490. https://doi.org/10.1161/01.CIR.81.2.482

Zeiher, A. M., Drexler, H., Wollschläger, H., & Just, H. (1991). Modulation of coronary vasomotor tone in humans: Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation*, *83*(2), 391–401. https://doi.org/10.1161/01.CIR.83.2.391

Zivanovic, Z., Divjak, I., Jovicevic, M., Rabi-Zikic, T., Radovanovic, B., Ruzicka-Kaloci, S., … Slankamenac, P. (2018). Association between Apolipoproteins AI and B and Ultrasound Indicators of Carotid Atherosclerosis. *Current Vascular Pharmacology*, *16*, 376–384. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8822346